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Monitoring bio-molecular vesicle interactions in liquid using Love-wave sensors

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Abstract

Surface acoustic wave micro-balances are particularly favourable for the development of biosensors. Their dimensions and physical properties offer a large potential in fluid investigations. We propose here a designed and manufactured innovating Love-wave sensor working in liquids for biological applications. This device based on delay line configurations is capable to detect and measure the presence of specific molecules on the sensing surface. Using this system, we managed to discriminate the vesicles spreading of the intact loading on sensor. This innovative SAW sensor reveals sensitive and reproducible device, capable to detect and quantify the biological interactions on sensing area.

Keywords: SAW sensors, bio-molecular interactions, lipid vesicles fusion

1. Introduction

Many researchers have initiated to design and manufacture sensors capable to detect and measure the presence of specific molecules in liquids for biological applications. Indeed, adsorption of proteins or lipid membranes onto inorganic substrates is an important issue yielding numerous developments in medicine and biotechnology [1-2]. Among these, the use of surface acoustic waves (SAW) has received a particular interest during the last decade. Specially, the use of pure shear guided waves in stratified substrates such as amorphous silica on quartz allowing for Love wave excitation appears as an attractive solution to fabricate devices able to operate in water. Since shear waves are not radiated in fluids and because of their noticeable sensitivity to gravimetric effects related to surface adsorption [3-4], they actually are considered as one of the best solutions for such applications. The first generation of SAW sensors developed in our group was based on Love wave resonators allowing detecting surface adsorption by monitoring the corresponding resonance frequency variations. Their functionality was illustrated by detecting bovine serum albumin (BSA) adsorption on the sensing area [5]. In the present work, we have developed SAW sensors based on delay line configurations found to be more compact at intermediate frequencies (125 MHz) and easier to implement. They exhibit a wide band acoustic signal not compatible with oscillator applications, but well suited for synchronous detection of phase and insertion loss changes. Furthermore, the device exhibits a large sensing area (3mm) enabling for well controlled liquid handling. In this paper, we

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show the efficiency of the sensor liquid cell and we demonstrate the large Love-wave signal dynamics when loading the device with fluids. The biological model we used consisted in lipid vesicles that behave differently function of the hydrophobicity of the sensing surface. Resulting phase shifts corresponding to intact lipid vesicles deposition and lipid vesicles fusion on silica and hydrophobic gold respectively have been clearly recorded. These encouraging results open perspectives for the study of more grafted complexes (protein or DNA) on biomimetic surfaces and in physiological conditions. Results show that phase shift allowed measurements and detection with high sensitivity of biomolecular interactions appearing in aqueous environment, in real time and without any labeling.

2. Materials and methods

2.1 Description of the Love Wave based microbalance

Our Love-wave devices consists in delay lines built on (AT,Z) cut of quartz ((YXlt)/36°/90° quartz cut). The wave guidance is achieved by depositing a 2.5µm thick silica overlay covered or not by 50 nm gold layer atop. The Love wave is excited and detected using IDTs composed of 50 pairs of 4-finger-per-wavelength electrodes made of 200 nm thick evaporated aluminum. The grating period is 10µm corresponding to a 40µm wavelength operating at 125 MHz. A 3.2 mm long cavity is achieved in between the two IDTs which corresponds to the location where biochemical reaction is assumed to take place. The acoustic aperture is 3.5 mm. Figure 1a&b describes the device.

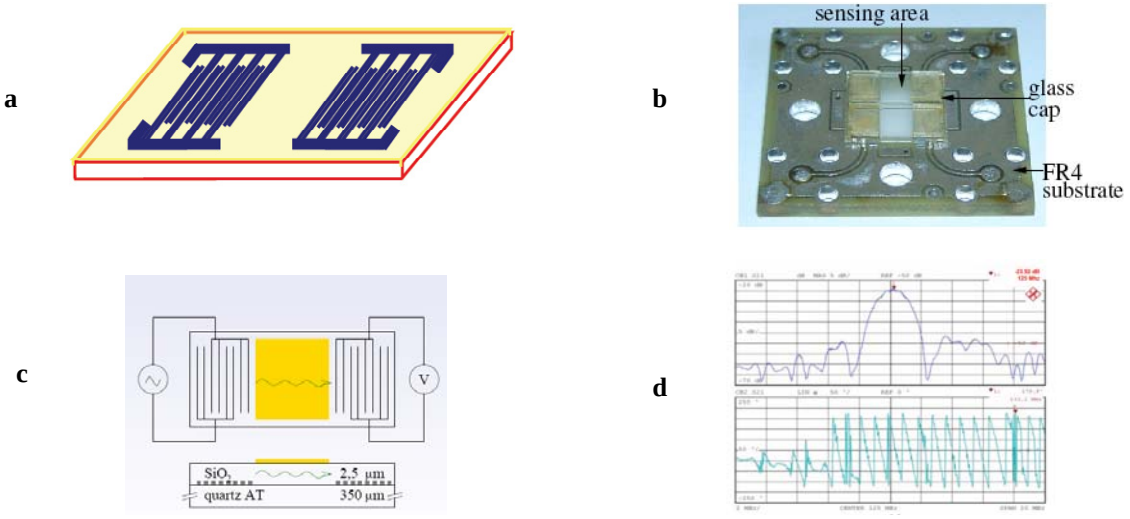

Fig 1: (a) Scheme of the Love-wave based delay line. (b) Photography of the dual delay line sensor equipped with specific PDMS cavities. (c) Scheme of the Love-wave device with electrodes. (d) Transfer function of delay line exploiting Silica guiding overlays.

The delay lines are fabricated using lift-off techniques to define the IDTs and aluminum evaporation. Figure 1 c&d shows the typical responses of Silica based Love-wave delay lines, showing the possibility to achieve insertion losses (IL) within the delay line pass band in the range 23-25 dB, previously described El Fissi et al. [6].

2.2 Biological and chemical materials

Small Unilamellar Vesicles (SUV) were prepared by sonication and extrusion at T = 25°C of a dimyristoylphosphatidylcholine (DMPC) lipid solution. Octadecyl-Mercaptan (OM) was used to functionalize the gold surface and give a hydrophobic monolayer, Octyl Gluco-pyranoside (OG) as a detergent to remove the lipid monolayer from the gold surface and sodium chloride to provoke an osmotic shock to take off adsorbed vesicles from the surface.

3. Results and discussion

3.1 Hydrophobic gold surface as sensing area.

A hydrophobic monolayer was realized on gold through functionalizing the surface in 1 mmol.dm^{-3} Octadecyl-Mercaptan at room temperature overnight as previously published [7]. We chose to monitor the lipid vesicles fusion or simple adhesion in real time on these surfaces, by recording changes in phase and magnitude signals at fixed frequency (125 MHz). First, preliminary experiments have been performed to evaluate the efficiency of the liquid cell and to check the Love-wave signal dynamics when loaded with fluids. The phase variation corresponding to the media change (liquid versus air) was achieved. As theoretically expected, the phase value decreases after loading the sensing area with liquid. We also confirm the reversibility of our device through successive steps of air or water exposition (figure 2a). In a second step, lipid vesicles were injected on sensing area (t = 2500s). Immediately, we can observe a more important negative phase shift, meaning that our sensor is able to detect the presence of vesicles in the solution. To check the stable surface adsorption of lipid vesicles, several water injections were realized. The phase value remained constant after this operation, and lower than in water, revealing a mass deposition on the sensing surface. We expected on hydrophobic surface the formation of a lipid monolayer obtained by vesicles fusion on the alkylated layer. Injection of the lipid vesicles induced a phase shift of around 2°. A lipid monolayer presents theoretically a mass of 150 ng.cm^{-2} [8]. Indeed, the DMPC lipid presents a molecular mass of 680 g mol^{-1} and its area is 70 Å^2. The sensor response should give a phase shift of 0.5° for a mass loading of 100 ng.cm^{-2}. During our experiment, 400 ng.cm^{-2} of lipids seemed to be deposited on the sensing gold area, conformably to the measured 2° phase shift. Thus, an excess of lipid material was deposited on the sensor. Generally a last step consists in a soft cleaning treatment, using for example diluted sodium hydroxide (NaOH 20 mmol dm^{-3}), to remove the excess lipid and to lead to a stable baseline. In our case, we didn't use any « cleaning » solution on hydrophobic surface just after lipid fusion on it. This probably explains the difference between our experimental data (400 ng cm^{-2}) and the expected theoretical value for a homogeneous lipid monolayer (150 ng cm^{-2}).

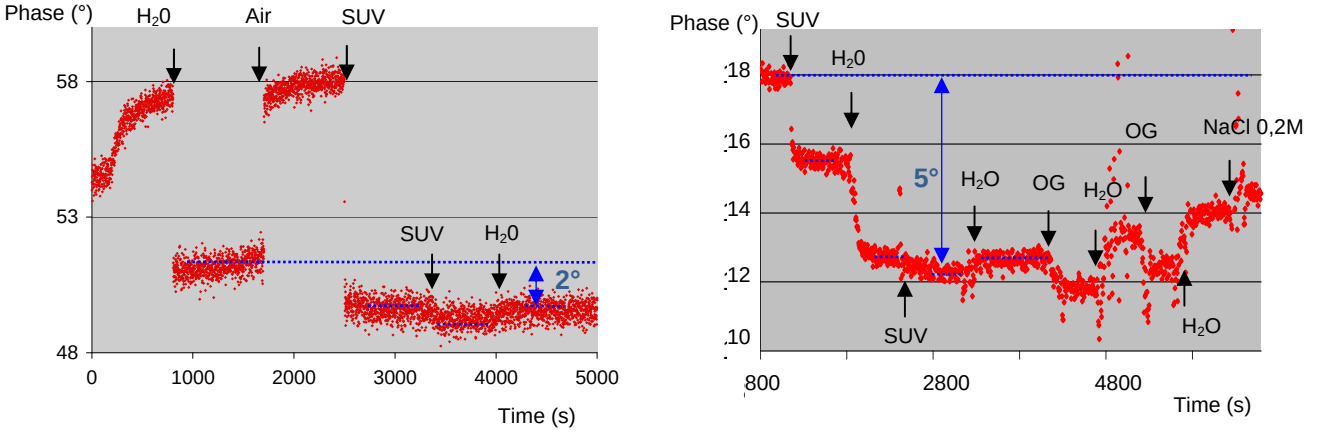

Fig 2: (a) Phase signal shift detected on sensor made of hydrophobic gold deposited on silica. Gold surface was functionalized with octadecyl-mercaptan (OM); (b) Phase signal shift detected on SAW sensor surface made of silica.

3.2 Hydrophilic silica surface as sensing area.

The hydrophilic surface was obtained without metal deposition on the sensor surface. The silica atop was directly used as a sensitive area. It is known that lipid vesicles adsorb but can be kept intact while presented to a hydrophilic surface [9]. Several behaviours of lipid vesicles are possible on hydrophilic surfaces, mainly depending on their oxidation state: 1) « non ruptured » lipid vesicles can adsorbed all the way towards saturated coverage, or 2) vesicles can initially adsorb in a « non ruptured » configuration but at a critical coverage, rupture is initiated and a supported lipid bilayer is formed. As for the previous experiment on gold surface, the phase variation corresponding to the media change (liquid versus air) was achieved (figure 2b). The first injection of small unilamellar vesicles induced a negative phase shift of 2° corresponding to a mass loading of 400 ng cm^{-2}, closed to the value expected for a lipid bilayer. Thus, the surface may have been partially covered by a lipid bilayer during this first lipid injection. The phase value remained constant after this step showing the stability of the lipid assembly on the surface. At t = 1800s, water injection was processed inducing immediately a 3° more decrease of phase value. First, it has to be specified that the lipid vesicles dynamics onto hydrophobic or hydrophilic surface is completely different. Lipid vesicles are highly attracted by hydrophobic surface leading to their fusion on it, while they are more stable in solution (less attracted by the surface) when presented to a silica substrate. In this last case, interaction between vesicles exists and this interaction competes with the lipid/surface interaction. Then, it establishes equilibrium between these two interaction phenomena. If the environmental conditions are modified (medium change for example), we can expect a displacement of this equilibrium, higher surface interactions leading to a phase shift. We effectively noticed a phase decrease after water injection, as if the dilution effect promoted the lipid vesicles interactions with the surface. A final phase shift of 5° was measured. The vesicles are delimited by a lipid bilayer and present a diameter of 100 nm. A layer of vesicles would then give a mass of 1300 ng cm^{-2}. We obtained a phase shift of 5° that corresponds to a mass of 1000 ng cm^{-2}. Thus, on our silica surface, after an injection of lipid vesicles followed by a washing step, we apparently achieved the formation of a continuous layer of intact vesicles.

Successive lipids injections were realized to check saturation of silica surface with lipid vesicles. The final step did consist in recovering the initial base line (before lipid injection) of the sensor, by successive cleaning operations with detergent ([OG] = 40 mmol dm^{-3}) and saline solution ([NaCl] = 0.2 mol dm^{-3}). Even if the surface regeneration was not completely obtained, the tendency was confirmed by regular phase increase at each injection of cleaning solutions.

4. Conclusion

The fusion of lipid vesicles on a surface was first described in the 1980s by McConnell et al. (1986) [10]. Lipid material can be transferred from the liposomes to both hydrophilic and hydrophobic surfaces. When liposomes hit a suitable solid surface, they may adsorb, break up, and spread to form a bilayer on a hydrophilic surface or a monolayer on a hydrophobic one.

Here, two types of sensing surfaces have been tested, either silica or silica covered with a gold film, surrounded with PDMS walls to allow operating in liquid. Our sensor was able to distinguish between the spreading of vesicles and the loading of intact lipid vesicles on the sensing surface. These encouraging results open the way for the development of well-controlled SAW-based biosensors, allowing for accurate analyses of specific molecule adsorption reactions.

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